Part III-C A one-page scientific abstract of the protocol

Canavan Disease is an autosomal recessive leukodystrophy caused by mutations in the aspartoacylase (ASPA) gene. The loss of ASPA activity leads to an elevation in the brain concentration of N-acetylaspartate (NAA), spongiform degeneration of oligodendrocytes, neurodevelopmental retardation and childhood death. Several features make it a good candidate for gene therapy and a prototypic neurogenetic disease for gene therapy experiments. These include: it is a autosomal recessive disease with defined mutations in a single gene; mutations result in a loss of function of the enzyme; pathology is restricted to the brain; no alternative treatments exist; it is uniformly fatal and morbidity is high; disease progress and CNS gene transfer efficacy can be followed non-invasively and quantitatively using proton NMRS of specific brain regions; only a small fraction of transduced cells might result in significant phenotypic effects. Alternative (non-ASPA) metabolites of NAA, specifically N-acetyl-aspartyl-glutamate (NAAG) are toxic. Lowering concentrations of NAA (and NAAG) should therefore limit brain injury. Rare patients with the juvenile form of Canavan Disease who have aspartoacylase deficiency and increased urinary NAA but normal cerebral NAA concentrations have no leukodystrophy, suggesting that the deficient cerebral NAA catabolism and not the enzyme mutation per se affects myelin metabolism. Sufficient expression of ASPA might lower regional NAA concentrations and enable partial phenotypic correction despite low transduction efficiency.

In this protocol we are using a non-viral vector, several components of which have previously been used in clinical trials, but other components which are novel and are designed to enhance expression in terminally differentiated cells of the brain. These components include: 1) a DNA plasmid, with the transcription unit flanked by AAV ITRs. The transcription unit includes the full-length human ASPA cDNA driven by the CMV promoter and with a SV40 polyadenylation signal; 2) Condensation of the DNA using a polycation, protamine. This condensation decreases the size of the plasmid-liposome complex and also helps prevent aggregation thereby facilitating diffusion through the brain; 3) The polycationic liposome, DC-Chol / DOPE to facilitate cellular uptake. This liposome has previously been used in 6 gene therapy trials.

The study includes neurological and quantitative psychometric evaluation and biochemical measures including regional analysis of NAA in frontal, parietal and occipital lobes using proton NMRS. Additional baseline evaluations include MRI using imaging parameters to obtain semi-quantitative myelin scores and VEPs, BAERs and SSER. The gene intervention phase includes a simple neurosurgical procedure with the implantation of a cerebrospinal fluid reservoir. The LPD complex will be delivered using the cerebrospinal fluid reservoir into the anterior horn of the right lateral ventricle, a delivery method based on experimental animal studies. The post-surgical phase includes repeated measurements using the same tests that were carried out prior to surgery during the baseline evaluation, repeated at different intervals post gene delivery. The retreatment phase may include the delivery of a higher dose of LPD/ASPA (10 ml) if a higher brain concentration of NAA is noted or there is evidence of loss of any expression noted, depending on evidence of safety as the trial progresses. The dose will depend on the level of endotoxin in the preparation, according to FDA guidelines and approval.